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Department of Biological Sciences, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.
E-mail: pnosila@sfu.ca

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Centrosome Duplication: Is Asymmetry the Clue?

The structure of the yeast Sfi1–centrin complex, and its asymmetric position within the yeast centrosome, suggest a model for the initiation of centrosome duplication and provides a target for licensing this event.

Michele H. Jones and Mark Winey

Centrosomes, the yeast equivalents of which are known as spindle pole bodies (SPBs), are microtubule-organizing centers of eukaryotic cells, which are made up of proteins but, like chromosomal DNA, are replicated in a tightly regulated manner that is coordinated closely with the cell division cycle. The mechanism of centrosome duplication is poorly understood, but insights are coming from studies of components such as centrin, a small calcium-binding protein that was first identified in the flagella of green algae [1]. Centrins have turned out to be ubiquitous, widely conserved proteins, now known from a variety of studies to be involved in assembly, and in some cases maintenance, of centrosomes, SPBs and the basal bodies of flagellae [2,3]. The involvement of centrin in multiple cellular processes, some calcium-dependent and some not, suggested that alternative binding partners would be discovered for the protein that define particular functions, and this has turned out to be the case. Several years ago, Kilmartin [4] uncovered a novel yeast protein called Sfi1 which binds centrin in the absence of calcium, is conserved in vertebrates and localizes to centrosomes in both yeast and vertebrates. Now, Kilmartin and colleagues [5] have reported a structural analysis of the Sfi1–centrin complex and its asymmetric arrangement in the SPB (Figure 1), the results of which suggest a plausible model for the initiation, if not the licensing, of SPB duplication.

Sfi1 contains approximately 20 repeats that are similar to a subset of IQ domains initially identified in unconventional myosins [6], where they were shown to mediate binding to calmodulin, another small, calcium-binding protein. Structures have been determined for two different co-crystals of Sfi1 repeats with bound centrin: one with two repeats crystallized in low calcium, and one with three repeats that required calcium to make suitable crystals [5]. Interestingly the structure is not significantly altered by calcium, suggesting little role for calcium binding by centrin in its interaction with Sfi1. The centrins on adjacent Sfi1 repeats interact in a head-to-tail manner, and some mutations that affect the interacting parts of centrin were found to disrupt function *in vivo* [5,7]. The Sfi1 repeats themselves are extended α helices, leading to the idea that Sfi1 is an elongated protein stabilized by centrin binding at one molecule per repeat. Li *et al.* [5] provided support for this stoichiometry by showing that a shortened Sfi1 containing only

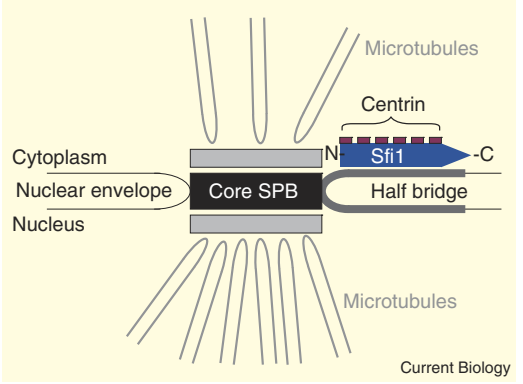

Figure 1. The Sfi1–centrin complex is positioned asymmetrically in the half-bridge of the yeast SPB.

The SPB is a trilaminar structure that lies in the nuclear envelope of the cell with microtubules emanating into the nucleus and the cytoplasm. Sfi1 (blue) with bound centrin (purple) is on the half-bridge with the amino terminus proximal to the core SPB and the carboxyl terminus distal.

15 repeats is bound by 15 centrin molecules, as measured by nondenaturing nanospray mass spectrometry; they also showed by electron microscopy that the complex forms a rod of predicted length.

Kilmartin [4] localized Sfi1 on the cytoplasmic face of the half-bridge adjacent to the SPB (Figure 1). The half-bridge is a modified region of the nuclear envelope that contains several membrane proteins as well as Sfi1–centrin [8]. The half-bridge is critical in SPB duplication because assembly of a new SPB begins on the cytoplasmic face of the half-bridge distal to the existing SPB [9]. The extended structure of centrin-bound Sfi1 is compatible with the length of the half-bridge (~90 nm). The size and the flat shape of the half-bridge suggest the presence of a number of parallel Sfi1–centrin complexes. Using immunoelectron microscopy in conjunction with two different allelic variants of Sfi1 tagged at either end, Kilmartin and colleagues [5] convincingly showed that the amino terminus of Sfi1 is adjacent to the SPB, whereas the carboxyl terminus lies at the distal end of the half-bridge. Hence, the asymmetric half-bridge structure contains an asymmetrically positioned molecular component. Moreover, the 'full bridge' that connects two duplicated spindle pole bodies before separation is about twice the length of the half-bridge. Interestingly, immuno-electron microscopy showed that this increase in length is due to an equivalent set of Sfi1 molecules placed as a mirror reflection, with the carboxyl termini of Sfi1 molecules in the middle and the amino termini at each SPB.

The asymmetric position of Sfi1 molecules in the half-bridge has important implications for SPB duplication. It is not widely appreciated that the initial event of duplication is bridge elongation, from half to full, and mutations that destroy bridge structure block duplication [8]. Furthermore, a demonstrated intermediate in SPB duplication has both a full bridge and the beginnings of a new SPB — called a satellite and shown to contain several SPB components — at the end of the bridge [9,10]. The structure and placement of Sfi1 [4,5] answers long-standing questions regarding the purpose of bridge elongation and the metric to determine bridge length. The model is that SPBs begin duplication by the addition of Sfi1–centrin complexes to the carboxy-terminal end of the Sfi1–centrin already present in the existing half-bridge. This may occur by direct interaction of the carboxyl termini of Sfi1 molecules, or it may be facilitated by another protein. After the full bridge containing the reflected Sfi1 molecules is assembled, then the amino terminus of the newly added Sfi1 becomes available to recruit core SPB components to assemble a new SPB. This step has been visualized by electron microscopy as the appearance of the satellite, on the cytoplasmic side of the nuclear envelope [9,10], in the same position on the bridge as the newly mapped amino terminus of Sfi1.

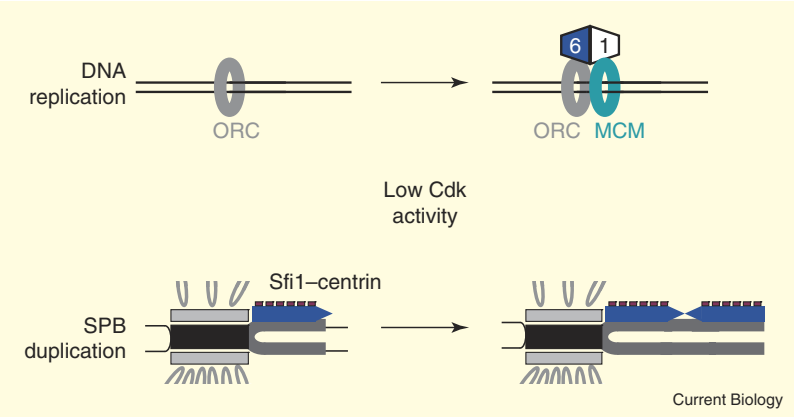

Figure 2. The licensing of SPB duplication may be regulated in a manner similar to yeast DNA replication.

Top: after DNA is replicated, it may not be replicated again until a number of conditions are met, including a period of low Cdk activity. This is called licensing and allows the assembly of the pre-replication complex. ORC, origin recognition complex; 6, Cdc6; 1, Cdt1; MCM, mini-chromosome maintenance. Bottom: a model for SPB licensing. After the SPB is duplicated, it may not be duplicated again until there is a period of low Cdk activity that allows for the addition of new Sfi1–centrin complexes (blue/purple) to the SPB.

The early and potentially critical role of Sfi1 in SPB duplication also makes it a candidate for licensing the event. The duplication of SPBs/centrosomes occurs once-and-only-once per cell cycle, like chromosomal DNA replication, and appears to have some common regulatory features. Low cyclin-dependent kinase (Cdk) activity upon exit from mitosis is critical for licensing chromosomal replication by allowing assembly of the pre-replication complex [11,12] (Figure 2, top). A similar regulatory event may occur at SPBs. Consistent with this idea, the inappropriate reduplication of yeast SPBs also requires a period of low Cdk activity [13]. In such a model, higher Cdk activity after SPB duplication prevents recruitment of Sfi1, but the drop in Cdk activity at mitotic exit allows for assembly of Sfi1 onto the half-bridge (observed as bridge elongation; Figure 2, bottom). Licensing may involve controlling the interactions of the carboxyl termini of Sfi1 molecules with each other or with other proteins. In any case, the localization of Sfi1–centrin and its role early in SPB duplication gives us a molecular framework for analysis.

The provocative nature of the Sfi1–centrin structure and localization makes outstanding questions about its precise function all the more pressing. For example, it will be informative to determine how the array of parallel Sfi1–centrin complexes is attached to the nuclear envelope, particularly since two of the membrane proteins at the half-bridge also bind centrin [8]. In addition, the function of the carboxyl terminus of Sfi1 is critical in models of Sfi1 function;

therefore, identifying factors that bind the carboxyl terminus and understanding how binding is regulated are critical questions. Finally, if Sfi1 assembly on the half-bridge is the initial event in SPB duplication, especially if that assembly is regulated as a licensing event, it will be very interesting to learn if these roles for Sfi1 are conserved during vertebrate centrosome duplication. Recent experiments suggest that a contributing factor in licensing centrosome duplication is the disengagement of the centrioles from each other at the end of mitosis [14,15]. It is likely that, as with DNA replication, a number of mechanisms will be uncovered that act together to protect the integrity of the genome, in this case, by ensuring the bipolarity of the mitotic spindle.

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MCD Biology, University of Colorado – Boulder, 347 UCB, Boulder, Colorado 80309-0347, USA.
E-mail: mark.winey@colorado.edu

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Inbreeding: When Parents Transmit More Than Genes

Inbreeding in wild populations can have devastating effects on fitness, but the genetic causes should not be transmitted across generations. A new study of song sparrows has revealed a parent–offspring resemblance for inbreeding, resulting from population structuring, with important implications for understanding the genetic causes of phenotypic variation in wild populations.

Marta Szulkin and Ben C. Sheldon

According to the standard model of inheritance, diploid sexual organisms inherit genes, not genotypes [1]. Genes inherited from each parent combine in offspring to produce genotypes, and thus the phenotypes, upon which natural or sexual selection acts. A resurgent theme in evolutionary biology at present is the use of various methods of genetic analysis, from quantitative genetics to functional genomics [2–4], to partition variation in the phenotypes observed in populations, and thus to understand the genetic causes of variation within and between populations. Very often, the focus in such analysis is on genes with additive effect, because such genetic effects are transmitted directly from parent to offspring, underlie the expected response to selection on a character [5], and are much easier to quantify than non-additive effects such as dominance or epistasis [1].

But phenotypes can also be influenced by interactions of genes from the two parents. These would seem to be of less interest for such analyses, for the simple reason that they cannot be transmitted directly to future generations. A recent study of inbreeding in song sparrows by Reid *et al.* [6], however, has come up with evidence for the apparent transmission of what can be treated as an interactive genetic effect. This finding challenges our assumptions about the methods that we use to explain variation in natural populations.

Inbreeding occurs when individuals that share a common ancestor mate. As we all share a common ancestor at some stage in our evolutionary history, we are all inbred in some sense. But it is 'close' inbreeding, such as matings between parents and offspring, or between siblings, that can have particularly dramatic effects on fitness, and it is the robustness and size of these effects — termed inbreeding depression — that presumably underlies incest avoidance in humans, and inbreeding avoidance in animals [7]. A number of long-term studies of wild mammal and bird populations have recently been able to quantify cases of close inbreeding between individuals known, from pedigrees, to be relatives [7–9].